JASEM ISSN 1119-8362

Full-text Available Online at www.ajol.info and www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *Sept.,* 2012
Vol. 16 (3) 291 - 294

Free Radical Scavenging Activities of Methanol Extract and Fractions of *Picralima nitida* (Apoceanacea)

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ABSTRACT: *Picralima nitida* commonly called picralima or pile plant is a tree or shrub with widely varied applications in West African folk medicine. The study evaluated the free radical scavenging activities of the crude methanol extract of *Picralima nitida* root bark and its various fractions. The methanol extract of *P. nitida* and its fractions were subjected to *in vitro* antioxidant evaluation using the DPPH free radical scavenging method. The IC-50 values for the percentage radical scavenging effects for the extract and fractions were determined. Statistical significance between the IC-50 values of the extract/fractions and that of ascorbic acid (standard) was determined using regression analysis. The crude extract has IC-50 value for radical scavenging activity of 5µg/mL which was significantly higher than that of ascorbic acid (2.55µg/mL). However, the percentage inhibition of the crude extract and ascorbic acid (standard) showed no significant difference. *Picralima nitida* has the potential for use as natural plant antioxidants in preventing against free radical damage. @JASEM

Key words: Picralima nitida, free radical, root bark extract.

Medicinal plants have been used in folk medicine for generations in most of the cultures throughout the world and are the primary form of treatment in many areas today. However, among the 250,000 – 500,000 species of plants on earth, only a relatively small percentage (1 – 10 %) is used for food by humans and animals (Borris, 1996). It is possible that more serve medicinal purposes. The medicinal values of plants have been claimed to lie in their phytochemical component including alkaloids, tannins, flavonoids and other phenolic compounds (Anyasor, 2011).

Free radicals such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated through endogenous processes such as metabolism, respiration and phagocytosis. They are also generated by exogenous systems such as pesticides, some pollutants, organic solvents and during radiation (Davies, 1995). However, the generation of these free radicals is normally balanced by an equivalent production of antioxidants through our natural antioxidant defense mechanism, which are the enzymatic antioxidants (superoxide dismutase, glutathione peroxidase, quinone reductase and catalases) and the non-enzymatic antioxidant (ascorbic acid, α-tocopherol, melatonin, β-carotene) obtained from the diet (Halliwell, 1996; Davies, 2000; Chun-Weng, 2011). However when the generation of free radicals overwhelm the antioxidant capacity of the biological defense system; it gives rise to oxidative stress (Zima, 2001). When oxidative stress occurred, it eventually leads to several deteriorating effects to our cellular bio-molecules such as DNA damage, lipid peroxidation, tissue injury and protein degradation (Chun-Weng, 2011).

Therefore, oxidative stress is increasingly recognized for their contribution to a number of diseases such as cancer, arthritis, neurodegenerative disorders, atherosclerosis and aging (Praveen and Awang, 2007). This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease (Atoui, 2005).

Nowadays, there is considerable interest in finding out about the antioxidants that are present in plants. Plants are the potential source of natural antioxidants that can protect against oxidative stress and therefore, have a main role to protect against injuries from lipid peroxidation. Carotenoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, polyphenols such as phenolics acids, flavonoids, proanthocyanidins, among others, are some of the antioxidants produced by plants for their survival (Praveen and Awang, 2007; Jimoh, 2008).

Picralima nitida (Stapf.) Th. & H. Durand is a West African plant belonging to the Apocynaceae family. It has widely varied applications in Ghana, Ivory Coast and Nigerian folk medicine. The seeds are crushed or powdered and taken orally, and are mainly used for the treatment of malaria (Kapadia, 1993) and diarrhoea. The seed, stem and roots have been reported to be effective as a cough suppressant, as well as an aphrodisiac and hypoglycaemic agent in treatment of diabetes (Ayensu, 1978). Studies have shown that it has some opioid analgesics activities (Menzies, 1998), some hypoglycaemic effects (Inya-Agha, 2006) and antimicrobial properties (Fakeye, 2004). There is apparently no scientific report on the antioxidant activity of the plant. The present study was designed to evaluate its free radical scavenging activity *viz a viz* the antioxidant capacity.

MATERIALS AND METHOD

Plant materials: Fresh *P. nitida* roots were collected in June, 2011 from a forest near Benin City, Nigeria. The plant material was identified and authenticated by the forest research institute of Nigeria, Ibadan where a herbarium specimen number 109429 was

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deposited. The roots were washed with water to remove earthy materials after which the bark was removed, air dried and powdered with the aid of a mechanical grinder.

Preparation of extract: Powdered plant material (3.2 kg) was extracted with 14 L of methanol by maceration at room temperature for two weeks. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extract was weighed and stored in an air-tight container and kept in the refrigerator at 4°C.

Fractionation of extract: The crude methanol extract was subjected to prefractionation/partitioning using different solvents. The crude extract (230 g) was first defatted with 7.5 L of petroleum ether; the ether insoluble portion was extracted with 16 L of chloroform followed by 7.5 L of ethyl acetate. The various fractions were concentrated to dryness, weighed and kept at 4°C in an air-tight container until free radical scavenging assay.

DPPH radical scavenging assay: The scavenging effect of crude methanol extract of *P. nitida* root bark and its various fractions on DPPH radical was estimated by method described by Jain *et al*., 2008. A solution of 0.1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0.01 – 0.2 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard. The ability to scavenge DPPH radical was calculated by the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC-50) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals (Jain, 2008).

Statistical analysis: The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range test using the Statistical Analysis System (SPSS Statistics 17.0).

RESULTS AND DISCUSSION

The IC-50 values of the methanol extract of *Picralima nitida* root bark and its fractions are shown in table 1. The IC-50 value 2.55 ± 0.06µg/mL obtained for ascorbic acid (standard) was significantly lower than the values obtained for the extract and fractions of *P. nitida*. The crude methanol extract has an IC-50 value of 5.30 ± 0.17µg/mL which is significantly lower than values obtained for the pet-ether and chloroform fractions (36.33 ± 2.08µg/mL and 8.73 ± 0.64µg/mL respectively)) but not significantly lower than that of ethyl acetate fraction (5.87 ± 0.12µg/mL.

Table 1: Evaluation of IC-50 values of extract and fractions of *P. nitida* root bark and ascorbic acid

Sample	IC-50 value (µg/mL)
Ascorbic acid	2.55 ± 0.06^{a}
Methanolic extract	5.30 ± 0.17^{b}
Pet-Ether fraction	36.33 ± 2.08^{c}
Chloroform fraction	8.73 ± 0.64^{d}
Ethyl acetate fraction	5.87 ± 0.12^{b}

Data represent mean ± Standard Deviation of triplicate analysis. Different lowercase letters within column indicate significant difference at $P \leq 0.05$.

Figure 1 shows the percentage radical scavenging activities of the extract and fractions. The extract and the various fractions exhibited appreciable percentage radical scavenging activities with the crudemethanol extract having values of 95.13 ± 0.62%, 95.32 ± 1.13%, 94.43 ± 0.45%, 93.13 ± 2.60%, 90.85 ± 1.24% at 10µg/mL, 25µg/mL, 50µg/mL, 100µg/mL and 200µg/mL respectively. These values were significantly higher than those obtained for the fractions. The scavenging activities of the extract and fractions were higher than 50% at the lowest concentration of 10µg/mL except that of the petroleum ether fractions which exhibited more than 50% scavenging activity at concentrations above 25 µg/mL. The interest in searching for natural antioxidants has increased considerably over the last few years. The present study estimated the DPPH free radical scavenging activity of the root bark of *P. nitida* as a measure of its antioxidant capacity.

A number of methods are available for the determination of antioxidant capacity but the assay involving the stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has received maximum attention due to its ease of use and its convenience (Sanchez-Moreno, 1998). The DPPH radical is one of the few stable organic nitrogen radicals, which bears a deep purple color. This assay is based on the measurement of the reducing ability of antioxidants toward DPPH. The ability can be evaluated by electron spin resonance (ESR) spectrometry or by measuring the decrease of its absorbance (Prior, 2005). The DPPH assay is considered to be mainly based on an electron transfer (ET) reaction, and hydrogen-atom abstraction is a marginal reaction pathway (Ou, 2005).

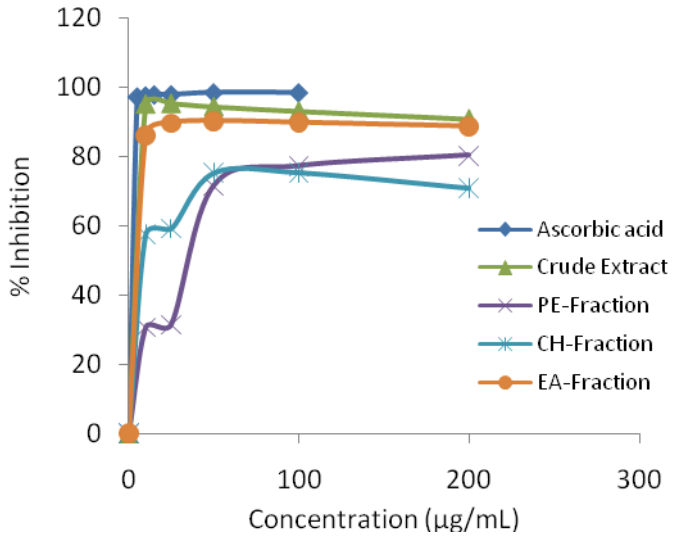

Fig. 1: DPPH radical scavenging activities of crude extract of *P. nitida* root bark and its fractions compared with ascorbic acid (standard)

The result of the DPPH radical scavenging assay showed that *P. nitida* extracts have appreciable DPPH scavenging effect with the crude extract being the most active. As shown in table 1, the 50% inhibitory concentration (IC-50) in the crude extract was significantly ($P < 0.05$) lower than the values obtained for the fractions except the ethyl acetate fraction. The extracts especially the crude and the ethyl acetate fraction have percentage scavenging effects that were comparable to that of ascorbic acid (the standard antioxidant) but these however were not statistically significant. In this study, we found a dose dependent relationship in the radical scanvenging activity of the petroleum-ether fraction of *P. nitida* extract.

The activity increased as the concentration increased for this fraction. This might be associated with the fact that the fraction is yet to achieve its maximum scavenging effects whereas the scavenging activities of the crude extract, chloroform and ethyl acetate fractions were non-dose dependent suggesting that they already exhibited their maximum scavenging effects at the concentrations used (fig. 1).

*Conclusion***:** The results of this study demonstrated that the root bark extract of *Picralima nitida* has good free radical scavenging activity which is a measure of its antioxidant capacity. Literature reports are evident that free radical scavenging ability of bioactive compounds is associated with antioxidant activity. Thus, we concluded that *P. nitida* can serve as a source of natural plant antioxidants. Ongoing work is focused on isolation and characterization of the bioactive compound(s).

Acknowledgement: The authors thank the department of Pharmaceutical chemistry, University of Benin for having providing the facility to carry out this research work. The authors would also like to appreciate the Federal Ministry of Education for the STEP-B/IOT award.

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